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(54) Title: PSORIASIS TREATMENT

(57) Abstract: This invention relates to the treatment of psoriasis by a NGF inhibitor, a composition comprising a NGF inhibitor, an animal model of psoriasis and a method of screening compounds for the treatment of psoriasis.

Psoriasis Treatment

Field

The present invention relates generally to the field of psoriasis and methods of treatment thereof.

Background

Psoriasis is a chronic scaling disease of the skin. Psoriasis is a noncontagious skin disorder that most commonly appears as inflamed swollen skin lesions covered with silvery white scale. This most common type of psoriasis is called "plaque psoriasis." It may range from just a few spots anywhere on the body to large areas of involvement. It does not spread from one part of the body to another or from one person to another. The cause of psoriasis is unknown.

Skin cells are programmed to follow two possible programs: normal growth or wound healing. In a normal growth pattern, skin cells are created in the basal cell layer, and then move up through the epidermis to the stratum corneum, the outermost layer of the skin. Dead cells are shed from the skin at about the same rate as new cells are produced, maintaining a balance. This normal process takes about 28 days from cell birth to death.

When skin is wounded, a wound healing program is triggered, also known as regenerative maturation. Cells are produced at a much faster rate, theoretically to replace and repair the wound. There is also an increased blood supply and localized inflammation. In many ways, psoriatic skin is similar to skin healing from a wound or reacting to a stimulus such as infection.

Lesional psoriasis is characterized by cell growth in the alternate growth program. Although there is no wound at a psoriatic lesion, skin cells (called "keratinocytes") behave as if there is. These keratinocytes switch from the normal growth program to regenerative maturation. Cells are created and pushed to the surface in as little as 2-4 days, and the skin cannot shed the cells fast enough. The excessive skin cells build up and form elevated, scaly lesions. The white scale

(called "plaque") that usually covers the lesion is composed of dead skin cells, and the redness of the lesion is caused by increased blood supply to the area of rapidly dividing skin cells.

The rapidly dividing skin cells have elevated levels of Nerve Growth Factor (NGF). NGF induces an inflammatory response, the proliferation of nerves and an upregulation of neuropepetides such as Substance P and calcitonin gene-related peptide. The elevated neuropeptide and NGF levels further induce the keratinocytes to proliferate. Thus, a vicious cycle of a proliferative and inflammatory process is established.

People who have psoriasis establish a better sense of control over their chronic disorder when they understand the range of treatment options available. It takes experimentation and persistence to discover effective treatments for an individual. Treatment success also requires faithful compliance to the regimen and a realistic understanding of psoriasis as a skin disorder. This is important because psoriasis is unpredictable. It has no cure and not all treatments work for each individual.

Treatments include topical formulations, systemic medications, and phototherapy. The severity of the psoriasis may guide the practitioner in deciding which treatment to use. Examples of topical formulations are steroid creams, retinoid lotions, and coal tar medications. These formulations can irritate the skin, make it more sensitive to the sun resulting in a tendency to burn, or cause the skin to thin. Phototherapy is the administration of ultraviolet light B or the drug Psoralent in combination with ultraviolet light A. Phototherapy is used when topical treatments have failed. Due to it's similarity to sun exposure, phototherapy poses a risk of skin cancer and skin aging. PUVA also has as a risk the development of cataracts. Finally, systemic medications include, for example, methotrexate, oral retinoids and cyclosporine. These agents are used when the psoriasis patient has not responded to other forms of treatment.

The treatments with little or no toxicity are used for mild psoriasis; the treatments with more side effects are used for moderate psoriasis. Treatments

with significant side effects are used for severe psoriasis. Thus, it is desirable to provide a treatment that has few side effects, is well tolerated by the patient and effective regardless of the severity of psoriasis.

In order to evaluate possible treatments for psoriasis an animal model would be helpful. The major pathological features of psoriasis are characterized by hyperproliferation and abnormal differentiation of keratinocytes; infiltration of inflammatory cells, angiogenesis and dilation of dermal blood vessels. Since there are no naturally occurring diseases in animals which exhibit all the phenotypic and immunological features of psoriasis, several approaches have been utilized which include studies on mutant strains of mice, development of transgenic mice and xenotransplantation models. Current animal models of psoriasis mimic various aspects of psoriasis but none present the complete pathology consistent with the features of psoriasis.

Prior to the development of transgenic mice, three spontaneous mouse mutations reflecting psoriasiform phenotypes, the flaky skin (*fsn*), chronic proliferative dermatitis (*cpd*) and homozygous asebia (*ab/ab*), mutant mice were used to study the pathophysiology of psoriasis. All these mutants display certain pathological features of psoriasis such as acanthosis, infiltration of mast cells and macrophages and increased vasculature of the dermis. However, the absence of T-cells in these infiltrates and ineffectiveness of Cyclosporine treatment suggest that these pathological features are not comparable to psoriasis.

The development of transgenics provided researchers a means to investigate the role of various factors in the pathogenesis of psoriasis. Transgenic mice overexpressing various cytokines in the epidermis lead to alteration of keratinocyte function generating psoriasiform phenotypes. Therefore transgenic animals with targeted expression of cytokine within the skin have been used to study the roles of these cytokines in the pathogenesis of psoriasis.

In transgenic mice which overexpress IL-1α and K14, there is infiltration of macrophages and monocytes within the dermis. In some cases inflammatory lesions are observed with acanthosis and parakeratosis. This model supports the

role of IL-1α as an inducer of inflammation. See Groves et al., PNAS 9:11874-8 (1995).

The IFNγ/involucrin transgenic mice shows hyperproliferation of keratinocytes. There is induction of MHC class II, ICAM-1 and enlarged dermal capillaries. Although there is infiltration of T-cells in the dermis, there is no epidermal T-cell infiltrate. See Carroll et al., J. Invest. Dermatol. 108:412-22 (1997).

In psoratic skin, VEGF has been implicated in angiogenesis. In VEGF/K14 transgenics there was an increased number of dermal mast cells and enhanced leukocyte adhesion and extravasation. Thus, VEGF may contribute to migration of inflammatory cells in the psoriatic lesions. See Detmar et al., J. Invest. Dermatol. 111:1-6 (1998).

Overexpression of leukocyte β2 integrins such as LFA-1 and MAX-1 in the PL/J mouse strain showed chronic skin inflammation, epidermal hyperplasia, hyperkeratosis, parakeratosis and lymphocyte exocytosis. See Bullard et al., PNAS 93:2116-21 (1996). Thus, this strain manifests several histological features typical for psoriasis but lacks the full symptomology.

Transgenic rats expressing human HLA-B27 and β2 microglobulin have also been reported to have several clinical and histological characteristics of psoriasis such as scales, erythema, dystrophic nails, acanthosis and lymphomorphonuclear cell infiltration. See Hammer et al., Cell 63:1099-1112 (1990).

However, as these models are created by manipulating a single gene, many times such models do not represent the phenotypes of these complex inflammatory diseases. Psoriasis being a polygenic disease it is unlikely to be truly reproduced in a model system by the manipulation of a single gene. The above-described transgenic models manifest various features of psoriasis, but none demonstrate the complete clinical and histological morphology characteristics of psoriasis.

Another approach to studying psoriasis has been the grafting of psoriatic plaques from patients with psoriasis onto genetically immunodeficient mice, i.e., xenograft models. The severe combined immunodeficient (SCID) mutation occurring in the BALB/c mouse prevents the development of mature B and T cells. The absence of a functional immune system allows xenogenic transplantation into SCID mice without major graft rejections. Symptomless skin from patients with psoriasis (non-lesional psoriatic skin) can be converted to a full-fledged psoriatic plaque skin by injection of autologous blood-derived immunocytes that have been stimulated with IL-2 and superantigens. Histologically, except for the occasional presence of a granular layer, transplanted lesions manifest the characteristics of psoriasis, such as hyperkeratosis, parakeratosis, intraepidermal microabscesses, suprapapillary thinning, angiogenesis and marked dermal infiltrates mainly composed of lymphomononuclear cells and dermal dendritic cells. Moreover, the immunocytes required stimulation by superantigens, a non-endogenous antigen, to induce the development of psoriatic plaques. Thus, this model fails to replicate the naturally occurring events of psoriasis.

The present invention provides for a method of treating psoriasis, a composition for the treatment of psoriasis, an animal model that replicates psoriasis without the use of non-endogenous stimulants, and a method of testing compounds for the treatment of psoriasis.

Summary

The present invention is directed to the treatment of psoriasis, especially targeting the inflammatory responses induced by NGF and its receptor system (NGF-R).

In one aspect of the invention there is a method comprising administering an effective amount of a NGF inhibitor to a patient in need thereof. In a preferred embodiment the NGF inhibitor is K252a. The NGF inhibitor may be administered subcutaneously, intralesionally, topically or systemically.

In a second aspect of the invention there is provided a composition comprising a theropeuticlally effective amount of a NGF inhibitor and a pharmaceutically acceptable carrier. In a preferred embodiment the NGF inhibitor is K252a.

In another aspect there is provided an animal model of psoriasis. Human psoratic non-lesional skin is grafted onto a SCID mouse to yield a SCID-HS mouse. Peripheral blood mononuclear cells (PBMC) are isolated from a patient suffering from psoriasis and stimulated with nerve growth factor (NGF) to yield stimulated PBMCs. The stimulated PBMCs are injected intradermally into the transplanted skin yielding an active psoratic plaque.

In yet another aspect there is provided a method of screening compounds for the treatment of psoriasis using the SCID-HS mouse. The prospective compound is administered to a transplanted psoriatic plaque and the effect on psoriatic features is observed. A decrease in histologic features indicates a possible therapeutic compound.

Description of the Figures

Figure 1 is a [Figure 1 from K252a paper].

Figure 2 is a [Figure 1 from NGF paper].

Figure 3 is a [Figure 2A & 2B from K252a paper].

Figure 4A & 4B is a psoriatic plaque before and after treatment with K252a, respectively.

Detailed Description

The invention will now be described in detail by way of reference only using the following definitions and examples. All patents and publications referred to herein are expressly incorporated by reference.

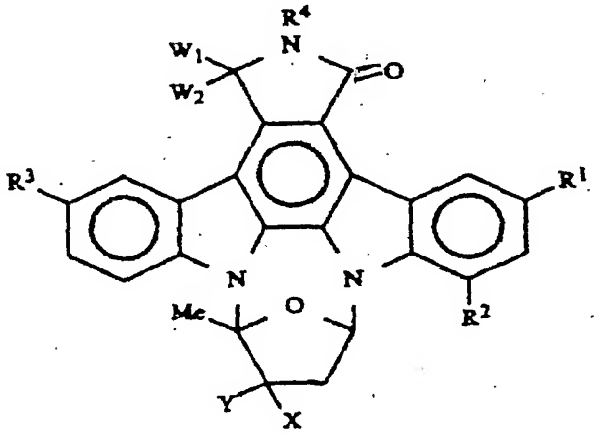
Definitions

As used herein, the following terms or abbreviations, whether used in the singular or plural, will have the meanings indicated:

"NGF" exists as a non-covalently bound homodimer that promotes the survival and growth of sympathetic, neural crest-derived sensory, and basal forebrain cholinergic neurons. NGF also is known to extensively interact with the endocrine system and in immune and inflammatory processes. (Reviewed in Scully and Otten, Cell Biol Int 19:459-469, 1995; Otten and Gadient, Int. J. Devl Neurosci 13:147-151, 1995 which are incorporated by reference).

The term "NGF inhibitor" means a molecule or moiety that prevents the action of NGF. The inhibitor may act directly, for example, by binding to the NGF receptor (NGF-R) preventing NGF from binding the receptor. The inhibitor may act indirectly, for example, by inhibiting the formation or release of NGF.

"K252a" is a member of a family of alkaloid toxins isolated from Nocardiopsis that specifically and potently inhibits NGF action, i.e., K252a is an NGF antagonist. K252a and its derivatives are represented by the formula (8R*, 9S*, 11S*)-(-)-9-hydroxy-9-methoxycarbonyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1H,8H, 11H-2,7b, 11a-triazadibenzo[a,g]cycloocta-[c,d,e,]trinden-1-one. The compounds are represented by formula (I):



Wherein W_1, W_2, R^1, R^2, R^3, R^4, X and Y represent various substituents. The compounds are physiologically active substances that inhibit protein NGF inhibitors and are useful as medicaments.

K252a was originally characterized as an inhibitor of protein kinase C and cyclic nucleotide-dependent kinases. Subsequently, K252a in nanomolar

quantities has been shown to be a specific inhibitor for NGF induced neurite outgrowth in PC12 cells. It has been reported that K252a inhibits the increase in c-fos oncogene transcription, the increase in intracellular calcium and the stimulation of the phosphorylation cascade produced by NGF in PC12 cells. Further it has been established that inhibition of the cellular effects of NGF by K252a is mediated by blocking trk proto-oncogene tyrosine phosphorylation and kinase activity.

The inventive compositions find use in the treatment of psoriasis. In a preferred embodiment the compositions are for topical administration. The products can be in the form of creams, lotions, liquids, powders, pastes, and the like, as would be appreciated by one of ordinary skill in the art. Regulating skin condition involves topically applying to the skin a safe and effective amount of a composition of the present invention. The amount of the composition which is applied, the frequency of application and the period of use will vary widely depending upon the level of the NGF inhibitor(s) and/or other components of a given composition and the level of regulation desired.

Lotions, gels and other topical application formulations

In general, most pharmaceutical creams and lotions contain oils, waxes, lanolins, sterols, humectants, emollients, thickening agents, proteins, preservatives, emulsifiers, silicones and the like as would be appreciated by one of ordinary skill in the art.

For application to the skin, the macro or microparticles of containing the NGF inhibitor(s) may be added to cosmetic compositions. The compositions may be provided in the form of gels, creams, lotions, solids, and other compositions, such as solutions and suspensions, aerosols or solid base or vehicle known in the art to be non-toxic and dermatologically acceptable to which a sufficient number of such particles are added under conditions in which the contents are released into the gels, creams, lotions, solids, solutions or suspensions, or aerosols. Upon application to the skin the gels, creams, lotions, solids, solutions or suspensions,

or aerosols may provide a method of administering the NGF inhibitor directly to the affected area or transdermal administration if systemic therapy is desired.

(1) **Lotions**

The lotions contain an effective concentration of a NGF inhibitor. Preferably, the reagents are encapsulated in a vehicle that releases its contents upon exposure to light or temperature, such that as the contents of the vehicle are released they provide a continuous administration of the NGF inhibitor. The effective concentration is that sufficient to produce the desired effect when contacting the skin. Any emollients known to those of skill in the art as suitable for application to human skin may be used. These include, but are not limited to, the following:

(a) Hydrocarbon oils and waxes, including mineral oil, petrolatum, paraffin, ceresin, ozokerite, microcrystalline wax, polyethylene, and perhydrosqualene.

(b) Silicone oils, including dimethylpolysiloxanes, methylphenylpolysiloxanes, water-soluble and alcohol-soluble silicone-glycol copolymers.

(c) Triglyceride fats and oils, including those derived from vegetable, animal and marine sources. Examples include. but are not limited to, castor oil, safflower oil, cotton seed oil, corn oil, olive oil, cod liver oil, almond oil, avocado oil, palm oil, sesame oil, and soybean oil.

(d) Acetoglyceride esters, such as acetylated monoglycerides.

(e) Ethoxylated glycerides, such as ethoxylated glyceryl monstearate.

(f) Alkyl esters of fatty acids having 10 to 20 carbon atoms. Methyl, isopropyl and butyl esters of fatty acids are useful herein. Examples include, but are not limited to, hexyl laurate, isohexyl laurate, isohexyl palmitate, isopropyl palmitate, isopropyl myristate, decyl oleate, isodecyl oleate, hexadecyl stearate, decyl stearate, isopropyl isostearate, diisopropyl adipate, diisohexyl

adipate, dihexyldecyl adipate, diisopropyl sebacate, lauryl lactate, myristyl lactate, and cetyl lactate.

(g) Alkenyl esters of fatty acids having 10 to 20 carbon atoms. Examples thereof include, but are not limited to, oleyl myristate, oleyl stearate, and oleyl oleate.

(h) Fatty acids having 9 to 22 carbon atoms. Suitable examples include, but are not limited to, pelargonic, lauric, myristic, palmitic, stearic, isostearic, hydroxystearic, oleic, linoleic, ricinoleic, arachidonic, behenic, and erucic acids.

(i) Fatty alcohols having 10 to 22 carbon atoms, such as, but not limited to, lauryl, myristyl, cetyl, hexadecyl, stearyl, isostearyl, hydroxystearyl, oleyl, ricinoleyl, behenyl, erucyl, and 2-octyl dodecyl alcohols.

(j) Fatty alcohol ethers, including, but not limited to ethoxylated fatty alcohols of 10 to 20 carbon atoms, such as, but are not limited to, the lauryl, cetyl, stearyl, isostearyl, oleyl, and cholesterol alcohols having attached thereto from 1 to 50 ethylene oxide groups or 1 to 50 propylene oxide groups or mixtures thereof.

(k) Ether-esters, such as fatty acid esters of ethoxylated fatty alcohols.

(l) Lanolin and derivatives, including, but not limited to, lanolin, lanolin oil, lanolin wax, lanolin alcohols, lanolin fatty acids, isopropyl lanolate, ethoxylated lanolin, ethoxylated lanolin alcohols, ethoxylated cholesterol, propoxylated lanolin alcohols, acetylated lanolin, acetylated lanolin alcohols, lanolin alcohols linoleate, lanolin alcohols ricinoleate, acetate of lanolin alcohols ricinoleate, acetate of ethoxylated alcohols-esters, hydrogenolysis of lanolin, ethoxylated hydrogenated lanolin, ethoxylated sorbitol lanolin, and liquid and semisolid lanolin absorption bases.

(m) Polyhydric alcohols and polyether derivatives, including, but not limited to, propylene glycol, dipropylene glycol, polypropylene glycol [M.W. 2000-4000], polyoxyethylene polyoxypropylene glycols, polyoxypropylene

polyoxyethylene glycols, glycerol, ethoxylated glycerol, propoxylated glycerol, sorbitol, ethoxylated sorbitol, hydroxypropyl sorbitol, polyethylene glycol [M.W. 200-6000], methoxy polyethylene glycols 350, 550, 750, 2000, 5000, poly(ethylene oxide) homopolymers [M.W. 100,000-5,000,000], polyalkylene glycols and derivatives, hexylene glycol (2-methyl-2,4-pentanediol), 1,3-butylene glycol, 1,2,6,-hexanetriol, ethohexadiol USP (2-ethyl-1,3-hexanediol), C15-C18 vicinal glycol and polyoxypropylene derivatives of trimethylolpropane.

(n) Polyhydric alcohol esters, including, but not limited to, ethylene glycol mono- and di-fatty acid esters, diethylene glycol mono- and di-fatty acid esters, polyethylene glycol [M.W. 200-6000], mono- and di-fatty esters, propylene glycol mono- and di-fatty acid esters, polypropylene glycol 2000 monooleate, polypropylene glycol 2000 monostearate, ethoxylated propylene glycol monostearate, glyceryl mono- and di-fatty acid esters, polyglycerol poly-fatty acid esters, ethoxylated glyceryl monostearate, 1,3-butylene glycol monostearate, 1,3-butylene glycol distearate, polyoxyethylene polyol fatty acid ester, sorbitan fatty acid esters, and polyoxyethylene sorbitan fatty acid esters.

(o) Wax esters, including, but not limited to, beeswax, spermaceti, myristyl myristate, and stearyl stearate and beeswax derivatives, including, but not limited to, polyoxyethylene sorbitol beeswax, which are reaction products of beeswax with ethoxylated sorbitol of varying ethylene oxide content that form a mixture of ether-esters.

(p) Vegetable waxes, including, but not limited to, carnauba and candelilla waxes.

(q) Phospholipids, such as lecithin and derivatives.

(r) Sterols, including, but not limited to, cholesterol and cholesterol fatty acid esters.

(s) Amides, such as fatty acid amides, ceramides, ethoxylated fatty acid amides, and solid fatty acid alkanolamides.

The lotions further preferably contain [by weight] from about 1% to about 10%, more preferably from about 2% to about 5%, of an emulsifier. The

emulsifiers can be nonionic, anionic or cationic. Examples of satisfactory nonionic emulsifiers include, but are not limited to, fatty alcohols having 10 to 20 carbon atoms, fatty alcohols having 10 to 20 carbon atoms condensed with 2 to 20 moles of ethylene oxide or propylene oxide, alkyl phenols with 6 to 12 carbon atoms in the alkyl chain condensed with 2 to 20 moles of ethylene oxide, mono- and di-fatty acid esters of ethylene oxide, mono- and di-fatty acid esters of ethylene glycol where the fatty acid moiety contains from 10 to 20 carbon atoms, diethylene glycol, polyethylene glycols of molecular weight 200 to 6000, propylene glycols of molecular weight 200 to 3000, glycerol, sorbitol, sorbitan, polyoxyethylene sorbitol, polyoxyethylene sorbitan and hydrophilic wax esters. Suitable anionic emulsifiers include, but are not limited to, the fatty acid soaps, e.g. sodium, potassium and triethanolamine soaps, where the fatty acid moiety contains from 10 to 20 carbon atoms. Other suitable anionic emulsifiers include, but are not limited to, the alkali metal, ammonium or substituted ammonium alkyl sulfates, alkyl arylsulfonates, and alkyl ethoxy ether sulfonates having 10 to 30 carbon atoms in the alkyl moiety. The alkyl ethoxy ether sulfonates contain from 1 to 50 ethylene oxide units. Among satisfactory cationic emulsifiers are quaternary ammonium, morpholinium and pyridinium compounds. Certain of the emollients described in preceding paragraphs also have emulsifying properties. When a lotion is formulated containing such an emollient, an additional emulsifier is not needed, though it can be included in the composition.

Other conventional components of such lotions may be included. One such additive is a thickening agent at a level from about 1% to about 10% by weight of the composition. Examples of suitable thickening agents include, but are not limited to: cross-linked carboxypolymethylene polymers, ethyl cellulose, polyethylene glycols, gum tragacanth, gum kharaya, xanthan gums and bentonite, hydroxyethyl cellulose, and hydroxypropyl cellulose.

The balance of the lotion is water or a C2 or C3 alcohol, or a mixture of water and the alcohol. The lotions are formulated by admixing all of the

components together. Preferably the NGF inhibitor(s) are suspended or otherwise uniformly dispersed in the mixture.

In certain embodiments the components may be mixed just prior to use. Devices for effecting such mixture are known to those of skill in the art or are exemplified herein.

(2) Creams

The creams are similarly formulated to contain an effective concentration typically at between about 0.1%, preferably at greater than 1% up to and greater than 50%, preferably between about 3% and 50%, more preferably between about 5% and 15% [by weight] of one or more of a NGF inhibitor provided herein. The creams also contain from about 5% to about 50%, preferably from 10% to 25%, of an emollient and the remainder is water or other suitable non-toxic carrier, such as an isotonic buffer. The emollients, as described above for the lotions, can also be used in the cream compositions. The cream may also contain a suitable emulsifier, as described above. The emulsifier is included is in the composition at a level from about 3% to about 50%, preferably from about 5% to about 20%.

(3) Solutions and suspensions for topical application

These compositions are formulated to contain an amount sufficient to produce a desired effect, for example, lessening of psoriatic symptoms, typically at a concentration of between about 0.1 - 10 mg/l preferably between 1 and 5 mg/l of a NGF inhibitor. The balance is water, a suitable organic solvent or other suitable solvent or buffer. Suitable organic materials useful as the solvent or a part of a solvent system are as follows: propylene glycol, polyethylene glycol [M.W. 200-600], polypropylene glycol [M.W. 425-2025], glycerine, sorbitol esters, 1,2,6-hexanetriol, ethanol, isopropanol, diethyl tartrate, butanediol, and mixtures thereof. Such solvent systems can also contain water.

Solutions or suspensions used for topical application can include any of the following components: a diluent, such as water saline solution, fixed oil, polyethylene glycol, glycerine, propylene glycol or other synthetic solvent; antimicrobial agents, such as benzyl alcohol and methyl parabens; antioxidants,

such as ascorbic acid and sodium bisulfite; chelating agents, such as EDTA; buffers, such as acetates, citrates and phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. Liquid preparations can be enclosed in ampules, disposable syringes or multiple dose vials made of glass, plastic or other suitable material. Suitable carriers may include physiological saline or phosphate buffered saline [PBS], and the suspensions and solutions may contain thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof. Liposomal suspensions, may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art.

These compositions that are formulated as solutions or suspensions may be applied to the skin, or, may be formulated as an aerosol or foam and applied to the skin as a spray-on. The aerosol compositions typically contain [by weight] from about 25% to about 80%, preferably from about 30% to about 50%, of a suitable propellant. Examples of such propellants are the chlorinated, fluorinated and chlorofluorinated lower molecular weight hydrocarbons. Nitrous oxide, carbon dioxide, butane, and propane are also used as propellant gases. These propellants are used as understood in the art in a quantity and under a pressure suitable to expel the contents of the container.

Solutions, may be formulated as 0.01%-10% isotonic solutions, pH about 4-8, with appropriate salts, and preferably containing one or more of the compounds herein at a concentration of about 0.1%, preferably greater than 1%, up to 50% or more. Suitable mild solutions are known to those of skill in the art. Such solutions, which have a pH adjusted to about 7.4, contain, for example, 90-100 mM sodium chloride, 4-6 mM dibasic potassium phosphate, 4-6 mM dibasic sodium phosphate, 8-12 mM sodium citrate, 0.5-1.5 mM magnesium chloride, 1.5-2.5 mM calcium chloride, 15-25 mM sodium acetate, 10-20 mM D.L.-sodium α-hydroxybutyrate and 5-5.5 mM glucose.

The active materials can also be mixed with other active materials, that do not impair the desired action, or with materials that supplement the desired action.

(4) Gels

Gel compositions can be formulated by admixing a suitable thickening agent to the previously described solution or suspension compositions. Examples of suitable thickening agents have been previously described with respect to the lotions.

The gelled compositions contain an effective amount of one or more an anti-hyperalgesic amount, typically at a concentration of between about 0.1 mg/l - 10 mg/l or more of one or more of a NGF inhibitor provided herein, from about 0.01% to about 75%, preferably from about 0.5% to about 20%, from about 1% to about 10% of the thickening agent; the balance being water or other aqueous carrier.

In another embodiment the compositions are for subcutaneous administration.

Pharmaceutical Preparations

The compounds of this invention are administered at a therapeutically effective dosage, i.e., that amount which, when administered to a mammal in need thereof, is sufficient to effect treatment, as described above (for example, to reduce or otherwise treat inflammation, pain and/or pyrexia in the mammal). Administration of the active compounds and salts described herein can be via any of the accepted modes of administration for agents that serve similar utilities.

The level of the inhibitor in a formulation can vary within the full range employed by those skilled in the art, e.g., from about 0.01% percent weight (%w) to about 99.99%w of the drug based on the total formulation. Preferably the inhibitor is present at a level of about 10%w to about 70%w.

Generally, an acceptable daily dose is of about 0.0001 to 150 mg per kilogram body weight of the recipient per day, preferably about 0.01 to 75 mg per kilogram body weight per day, and most preferably about 0.1 to 30 mg per kilogram body weight per day. Thus, for administration to a 70 kg person, the dosage range would be about 0.007 mg to 10.5 g per day, preferably about 0.7 to 5.25 g per day, and most preferably about .070 mg to 2.1 g per day.

Administration can be via any accepted systemic or local route, for example, via parenteral, oral (particularly for infant formulations), intravenous, nasal, transdermal or topical routes, in the form of solid, semi-solid or liquid dosage forms, such as for example, tablets, suppositories, pills, capsules, powders, solutions, suspensions, aerosols, emulsions or the like, preferably in unit dosage forms suitable for simple administration of precise dosages.

The compositions will include a conventional pharmaceutical carrier or excipient and at least one of a NGF inhibitor(s) and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, etc. Carriers can be selected from the various oils, including those of petroleum, animal, vegetable or synthetic origin, for example, peanut oil, soybean oil, mineral oil, sesame oil, and the like. Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. Other suitable pharmaceutical carriers and their formulations are described in "*Remington's Pharmaceutical Sciences*" by E. W. Martin.

If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc.

The compounds of this invention are generally administered as a pharmaceutical composition which comprises a pharmaceutical excipient in combination with at least one NGF inhibitor. The level of the inhibitor in a formulation can vary within the full range employed by those skilled in the art, e.g., from about .01% percent weight (%w) to about 99.99%w of the drug based on the total formulation and about .01%w to 99.99%w excipient. Preferably, the

formulation will be about 3.5 to 60% by weight of the pharmaceutically active compound, with the rest being suitable pharmaceutical excipients.

Intravenous Administration

In yet another embodiment, the compositions are for systemic administration.

Intravenous injection has proven to be an important route of administration for antiviral agents. The compounds of the present invention can be administered via this route, for example, by dissolving the compound, salt, ester or ether in a suitable solvent (such as water or saline) or incorporation in a liposomal formulation followed, by dispersal into an acceptable infusion fluid. A typical daily dose of a compound of the invention can be administered by one infusion, or by a series of infusions spaced over periodic intervals.

Examples

The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

Example 1

Transplantation of normal skin and psoriatic plaque to the SCID mouse

4-6 weeks old BALB/cByJSmn-*Prdkc*^{scid}/*J* SCID mice of either sex were purchased from The Jackson Laboratory, Bar Harbor, ME and maintained in Stanford University Research Animal facility. These mice were housed at this facility in a pathogen-free environment. At 6-8 weeks of age the mice were used for transplantation. Initially, they were anesthetized with intra-peritoneal injection of 30 mg/kg body wt. Ketamine-HCl and 1 mg/kg body weight and sedated with Acepromazine. After anesthesia, mice were prepared for transplantation by shaving the hair from the dorsal skin, 2 cm away from the head. This area was then sterilized and cleaned with providin iodide and alcohol. Graft beds of approximately 1 cm^2 were created on the shaved areas by removing full

thickness skin down to the fascia. Partial thickness human skin and shave biopsies from psoriatic plaques were then orthotropically transferred onto the graft bed. The transplants were held in place by gluing the human skin to mouse skin with Nexaband liquid, a veterinary bandage (Veterinary Products Laboratories, Phoenix, AZ). Finally the transplant and the wounds were covered with thick layer of antibiotic ointments. After 3-4 weeks of transplantation a 2 mm punch biopsy was obtained to confirm the acceptance of the graft and the origin of the skin in the transplant area. The normal human skins were obtained from elective plastic surgeries and psoriatic plaques were obtained from shave biopsies from psoriatic volunteers. The use of such tissues were approved by Santa Clara Valley Medical Center, Medical Review Board, California. Partial thickness skin was prepared by dermatome shaving of the skin and transplanted to the mouse as described above for the psoriatic plaque.

In each mouse acceptance of the graft was confirmed by histological and immunohistochemical staining. Figure 1 clearly demonstrates that the human skin is positively stained with HLA-ABC antibody whereas the adjacent mouse skin is negative. Four weeks after transplantation the xenografts appeared like normal skin (see Fig. 2). There were no scales, erythrema or acanthosis in the skin.

Compared to the normal human skin grafts the number of regenerated terminal cutaneous nerves positive for NGF-R were significantly higher in the transplanted plaques (see Figure 3). This corroborates the in vivo effect of the increased amounts of NGF from the keratinocytes of psoriatic plaques.

Example 2

Isolation and stimulation of PBMC's

Autologous peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by Ficoll-Histopaque (Sigma, St. Louis, MO) density centrifugation. 2 X 10^6 PBMC/ml were cultured on 24 well cell culture plates in complete media containing 10 % heat-inactivated autologous serum in RPMI-1640 (Sigma, St. Louis, MO), supplemented with 100 μg/ml streptomychin (Sigma, St. Louise, MO) and 50 μg/ml gentamicin (Sigma, St. Louise, MO).

Cells were cultured with 1 nM NGF with 20 U/ml IL-2, or 1 μg/ml each of *Staphylococal enterotoxins* (SE) SEB and SEC with 20 U/ml IL-2 or 20 U/ml IL-2 alone for 48 hours at 37° C in a humidified atmosphere containing 5 % CO_2. After incubation cells were removed from the plates, washed twice in RPMI-1640 and resuspended in sterile PBS.

Example 3

Injection of cells and reagents to the SCID mouse to convert non-lesional skin to psoriatic plaques

Shave biopsies were taken from non-lesional skins of psoriatic patients then orthotropically grafted on the SCID mouse. The mice were used for experiments 3-4 weeks after human skin transplantation. Only those mice whose grafts grossly showed no sign of inflammation or rejection were used. 2 mm punch biopsies were collected at the end of the 4th week of transplantation to confirm the status of the graft.

The mice were divided into three groups. One group was injected PBMC stimulated with 1 nM NGF and 20 U/ml IL-2, the second group was injected with PBMC stimulated with 1 μg/ml each of *Staphylococal enterotoxins* (SE) SEB and SEC and 20 U/ml IL-2, and the the third group was injected with PBMC stimulated with 20 U/ml IL-2 alone. Autologous cells along with 100 pg IFN-γ diluted in sterile PBS were injected intradermally (300 μl) into the xenograft. Injections were given once in a week for 3 weeks.

One week after 3 injection of autologous PBMC stimulated with 1 nM NGF and 20 U/ml IL-2 over a period of 3 weeks the xenograft became scaly with marked erythema. The thickness of the transplant was increased (Fig 2). Similar phenomena were observed with injections of PBMC stimulated with. *Staphylococal enterotoxins* (SE) SEB and SEC and 20 U/ml IL-2 (Fig. 2). On the contrary, the xenotransplant injected with IL-2 stimulated PBMC did not convert to psoriatic plaque.

Example 4

Collection of biopsies

2 mm punch biopsies were collected at before the injection of cells and also 1 week after the last injection. Some biopsies were taken at the mouse and human skin junction to assess the effects on the mouse skin. Immediately after the biopsies were taken, the skin tissue were embedded in Tissue Freezing Medium (Triangle Biomedical Sciences, Durham, N.C.) and snap frozen in liquid nitrogen. Finally, cryosections were prepared from the biopsies, fixed in cold acetone for 10 minutes, stained with hematoxylin and eosine and evaluated for the effect of differentially stimulated PBMC on the xenograft.

Example 5

Immunoperoxidase Staining for HLA-ABC and HLA-DR

6-μm serial sections were cut from the frozen specimens and stored in -20° C until use. At the time of immunostaining the sections were brought to room temperature and fixed in cold acetone for 5 minutes and washed with 0.01 M PBS for another 5 minutes. The sections were initially incubated either with mouse monoclonal anti-HLA-ABC antibody (1:100 dilution, Immunotech, Westbrook, ME) or with mouse monoclonal anti-HLA-DR antibody (1:100 dilution, Dako Corporation, Carpinteria, CA) for 18 hours at 4° C. All antibodies were diluted in Tris-antibody diluent buffer (Dako Corporation, Carpinteria, CA). After washing, the sections were incubated for 30 minutes at room temperature with biotinylated secondary antibody (horse anti-mouse IgG, 1:200 dilution, Vector Laboratories Inc. Burlingame, CA). Following this, the sections were incubated in avidinbiotin peroxidase complex (1:100 dilution, Vector Laboratories Inc. Burlingame, CA) in PBS for 20 minutes at room temperature. For visualization of antigen-antibody complex, the sections were treated with 0.03% diaminobenzidine tetrahydrocholoride (Vector Laboratories Inc., CA), and 0.07% hydrogren peroxide in acetate-imidazole buffer (pH 7.4) for 30 seconds. Finally, the sections were rinsed in phosphate buffer, dehydrated in ethyl alcohol, cleared and mounted on slides in DPX and observed under microscope.

Example 6

Immunofluorence Staining for NGF-R and CD8$^+$T Cells

14-μm serial sections were cut from the frozen specimens and were fixed and processed as described in the previous section. The sections were incubated with mouse monoclonal anti-NGR receptor antibody (1:50 dilution, Bhoringer Manheim, Germany) or anti-CD 8^+ T cells for 18 hours at 4° C. A secondary FITC conjugated horse anti-mouse IgG (1:100 dilution, Dako Corporation, Carpinteria, CA) was used at 1:200 dilution for visualization. The sections were incubated with the secondary antibody for 1 hour at room temperature and the samples were observed under a fluorescent microscope.

Example 7

Treatment of the transplanted psoratic plaques with K252a in SCID mouse model

The mice were used for experiments 3-4 weeks after human skin transplantation. Only those mice whose grafts grossly showed no sign of inflammation or rejection were used. Two millimeter punch biopsies were collected at the end of the fourth week of transplntation to confirm the status of the graft.

The mice were divided into two groups, the treatment and the control group. In the treatment group, the transplanted lesions were treated with 100 μg/kg body weight/day K252a in normal saline for 14 days. Daily doses were divided into two injections of 150 μl each and a total of 300 μl volume was administered intralesionally to the plaques. In control mice, 300 μl of saline was administered in two injections daily. One week after the 14 day regimen biopsies were collected from the transplants of both treatment and the control groups. Immediately after the biopsies were taken, the skin tissue was embedded in Optimum Cutting Temperature compound (OCT) and snap frozen in liquid nitrogen. Finally, cryosections were prepared, and evaluated for the effect of K252a.

Transplanted plaques treated with K252a had significant clinical and histological improvement compared to the controls. Lesions treated with K252a following three weeks of treatment had reduced scales, erythema and infiltration. Histological improvement in the treated plaques was evidenced by marked reduction of hyperkeratosis, acanthosis and reduced number of lymphomononuclear cellular infiltrates (Figures 4A & 4B). Compared to the length of the rete pegs of 308.57 ± 138.72 μm and 164.64 ± 64.78 μm before and following treatment with K252a, the respective numbers in the controls were 269.37 ± 57.78 μm and 209.37 ± 74.00 μm (Table 1).

Group	Before Treatment	After Treatment
K252a (100 μg/kg body wt. for 14 days)	308.57 ± 138.72 μm	164.64 ± 64.78 μm[1]
Control	269.37 ± 57.78 μm	209.37 ± 74.00 μm

1. $p < 0.05$

K252a was found to be therapeutically effective in both transplanted psoriatic plaques and converted plaques of non-lesional psoriatic skin. Subsequent to treatment with K252a, in addition to the histological changes in the H & E staining there was marked reduction of HLA-DR positive lymphocytes in the dermis and $CD8^+$ T cells in the epidermis.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

Claims

1. Use of a SCID-HS mouse as an animal model for the purpose of testing compounds for the treatment of psoriasis.

2. Use of claim 1 wherein, said testing comprises: (1) grafting human psoriatic non-lesional skin onto said mouse and injecting peripheral blood mononuclear cells (PBCM) stimulated with nerve growth factor (NGF) intradermally into the transplanted skin.

3. Use of claim 1 or claim 2 wherein the test compound is an NGF inhibitor.

4. Use of claim 3 wherein the NGF inhibitor is K252a.

5. Use of an NGF inhibitor in the preparation of a medicament for the treatment of psoriasis.

6. Use according to claim 5 wherein the NGF inhibitor is K252a.

7. A composition for the treatment of psoriasis comprising an therapeutically effective amount of an NGF inhibitor and a pharmaceutically acceptable carrier.

8. The composition of claim 7 wherein the NGF inhibitor is K252a.

9. The composition of claim 7 or 8 wherein, the NGF inhibitor is administered subcutaneously, intralesionally, topically or systemically.

10. The composition of claim 9 wherein the NGF inhibitor is administered intralesionally.

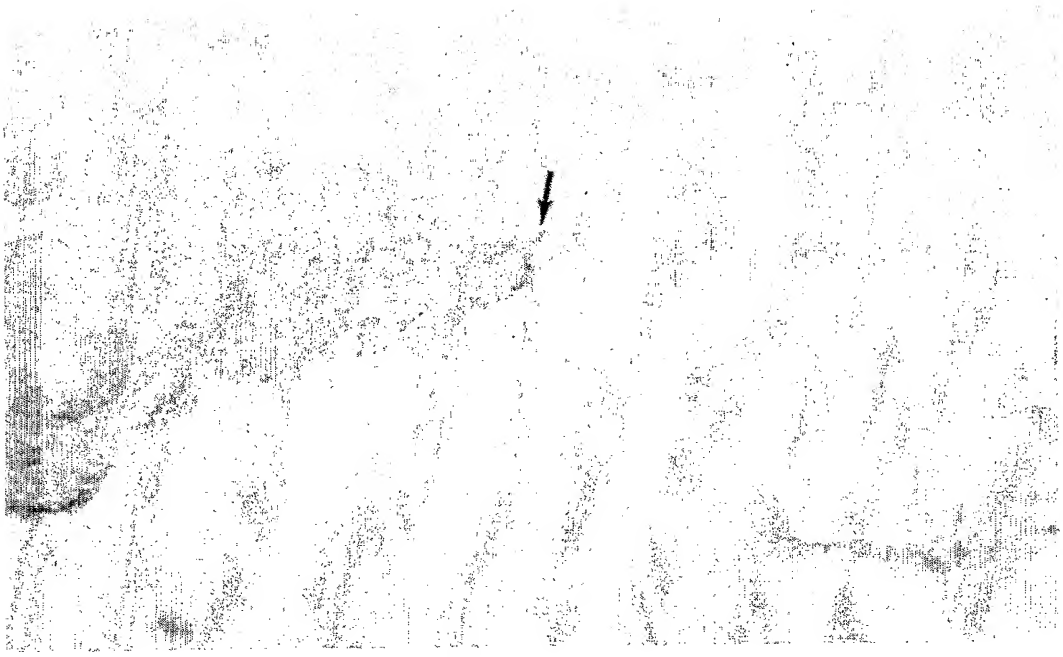


Figure 1

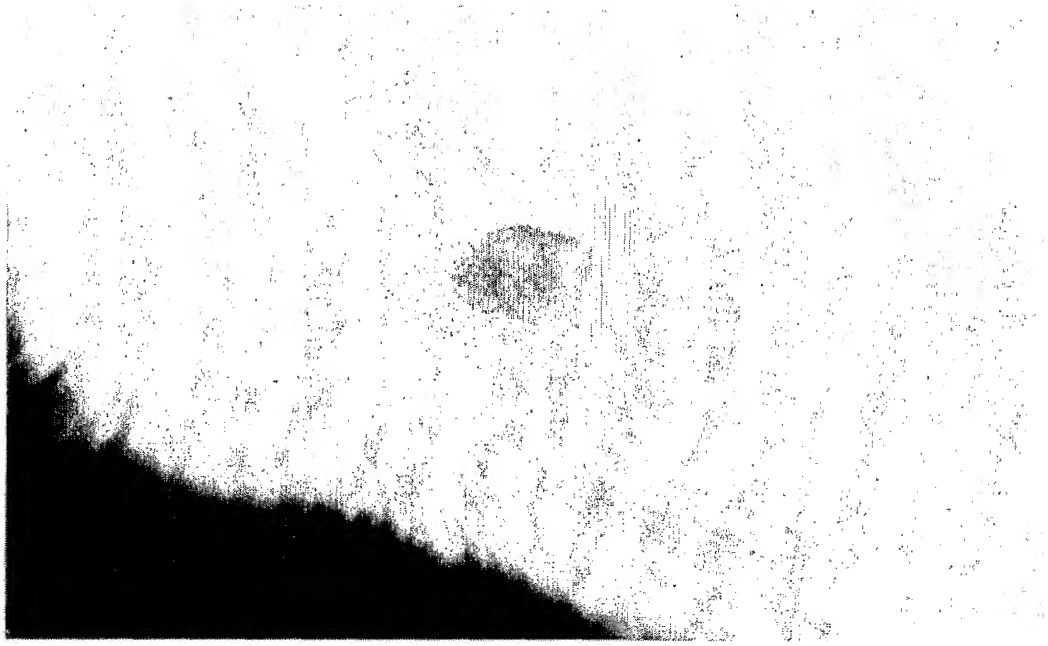


Figure 2(A)

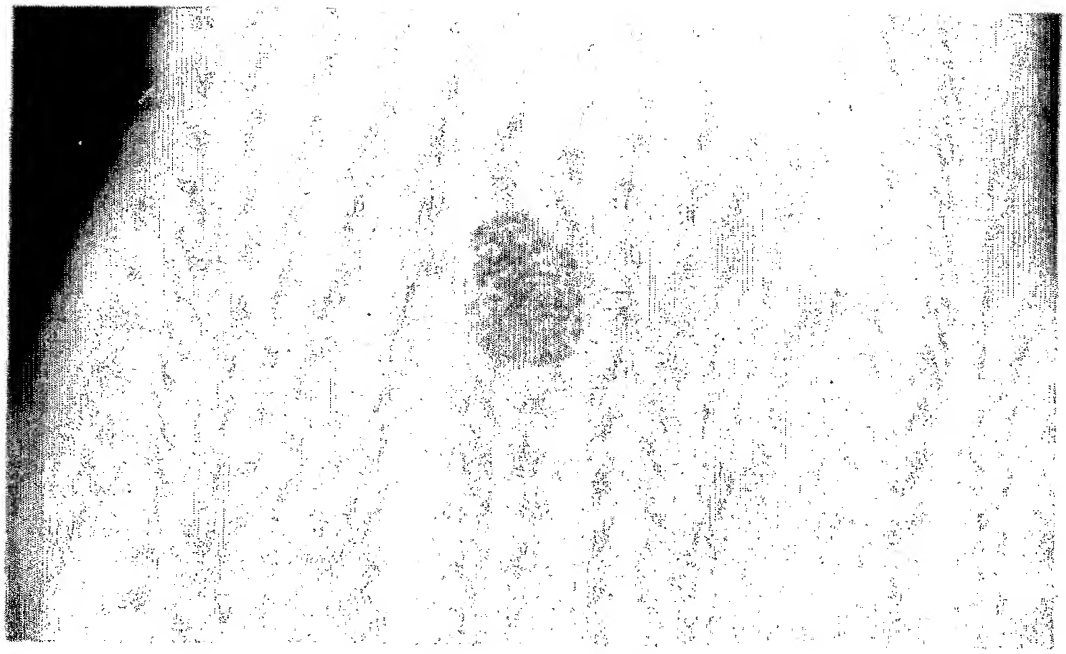


Figure 2(B)

Figure 3(A)

Figure 3(B)

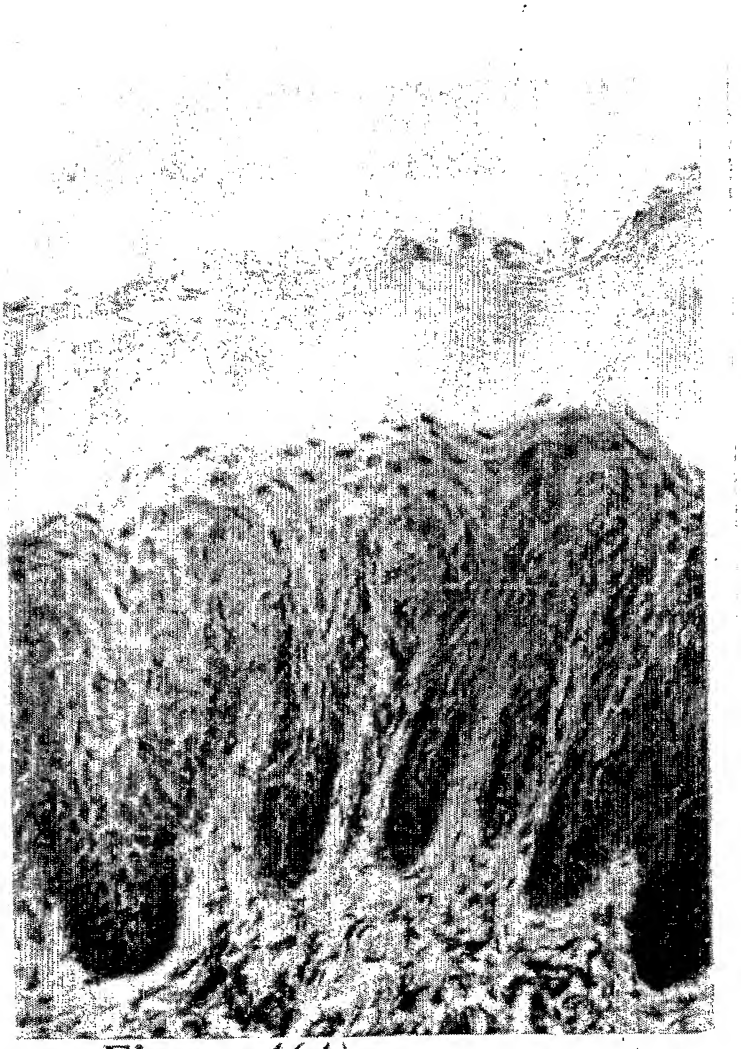


Figure 4(A)

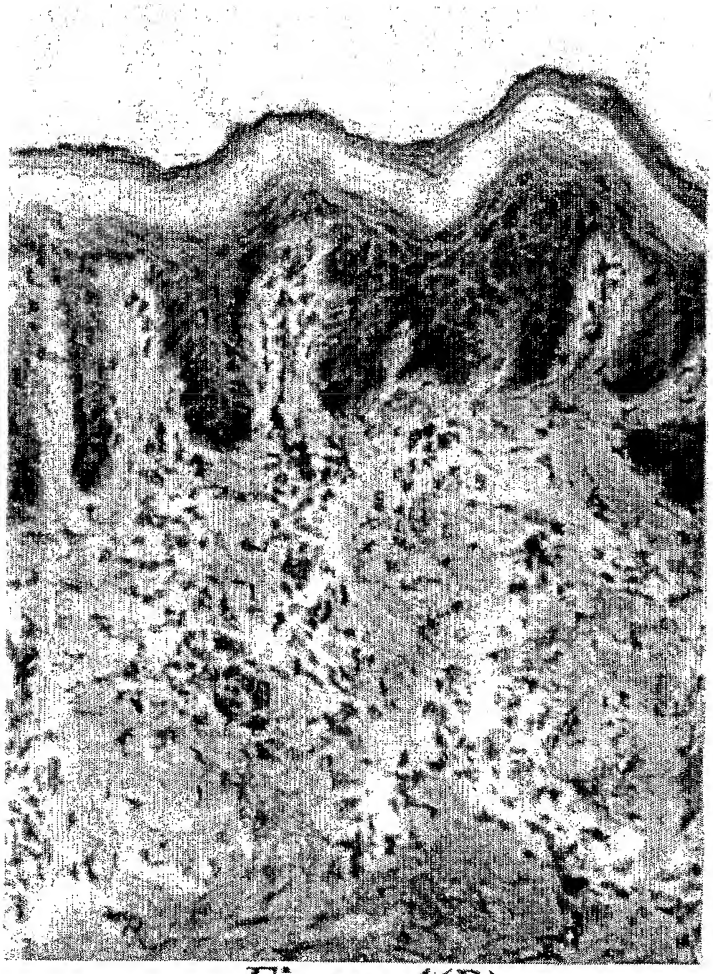


Figure 4(B)